An Automated Method to Quantify Radiation Damage in Human Blood Cells

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Abstract

A clinical cytogenetics karyotyping software system was used to evaluate the frequency of stable and unstable chromosome aberrations in peripheral blood lymphocytes of former radiation workers with internal deposits of plutonium. In a previous study of these workers based on "painting" chromosomes 1, 4 and 12, the fluorescence in situ hybridization (FISH) method showed elevated rates of chromosome translocations decades after plutonium intakes. The frequency of total translocations was also found to correlate with the bone marrow dose.[1] In cases with surplus, unstained slides from the earlier FISH study, the karyotyping method based on conventional (solid) Giemsa staining yielded a total translocation frequency estimate comparable to the FISH study. In contrast, the karyotyping method produced a significantly higher frequency of dicentric chromosomes than the FISH method (pancentromeric staining) which appeared to underestimate the true frequency. The cause of the discordant result is unknown. Overall, when comparing the mean aberration rate of the control (n=15) and exposed groups (n=15), the effect of the radiation exposure was detected by automated karyotyping of as few as 50 cells per individual.

Introduction

Cytogenetic analysis of blood lymphocytes is a well established method to assess the absorbed dose in persons exposed to ionizing radiation. Because mature lymphocytes circulate throughout the body, the dose to these cells is believed to represent the average whole body exposure. Cytogenetic methods measure the incidence of structural aberrations in chromosomes as a means to quantify DNA damage which occurs when ionizing radiation interacts with human tissue. Methods to quantify DNA damage at the chromosomal level vary in complexity and tend to be laborious and time consuming. In a mass casualty scenario involving radiological/nuclear materials, the ability to rapidly triage individuals according to radiation dose is critically important. For high-throughput

screening for dicentric chromosomes, many of the data collection steps can be optimized with motorized microscopes coupled to automated slide scanning platforms.

This study describes the application of an automated karyotyping system to measure DNA damage in lymphocytes by quantifying the prevalence of structural chromosome aberrations (both total translocations and dicentrics). To evaluate the method we used surplus, unstained metaphase preparations previously collected from retired plutonium workers with known radiation doses associated with internal depositions of plutonium. The original cytogenetic study of the workers utilized a chromosome painting method (FISH) and surplus slides from this study were used for a follow-up evaluation based on automated karyotyping.[1]

Methods

The karyotyping method involves conventional (solid) Giemsa staining combined with digital imaging and an automated clinical karyotyping software system known as BandView (Applied Spectral Imaging). Unlike most FISH-based methods, the karyotyping approach screens the entire human genome by capturing all 23 pairs of chromosomes based on Giemsa staining, digital imaging and automated karyotyping. Digital images of metaphase cells are captured and processed electronically using the specialized cytogenetic software. The chromosomes are automatically sorted and classified by size, including length, and centromeric index (the ratio of the length of the short arm to the whole length of the chromosome) in accordance with the International System for Cytogenetic Nomenclature (2005).[2] When the chromosomes are stained using methods that do not produce bands, they can still be classified into seven readily distinguished groups (A 1-3, B 4-5, C 6-12, D 13-15, E 16-18, F 19-20, G 21-22) of autosomes plus the XY sex chromosomes. In a metaphase cell with a full complement of 46 chromosomes, any deviation from the normal chromosome number for any of the seven groups signifies the presence of a structural rearrangement. Also, any morphologic disparity in the relative lengths of the short and long arms of the members of a pair denotes a structural anomaly. The analysis of karyotypes as a means to detect radiation-induced chromosome aberrations was reported by Nakano et al. (2001) in a study of A-

bomb survivors which compared FISH – based results with previous conventional Giemsa staining on the same 230 individuals.[3] Although conventional photographic methods and manual karyotyping were used, rather than electronic karyotyping, results showed that the earlier conventional Giemsa staining method detected a mean translocation frequency that was 73% of the genome equivalent frequency as detected by FISH. Details of the manual karyotyping process are described in a manual published on the Radiation Effects Research Foundation website by Awa[4] and cited in the Nakano et. al, paper.[3]

Deviations from the normal chromosome number assigned to each group and/or changes in short and long arm lengths between homologous members of a chromosome pair reveal many types of structural alterations which are detected microscopically, digitized and displayed on the computer monitor. Thus both symmetrical aberrations (translocations and inversions) and asymmetrical aberrations (deletions, dicentrics and acentrics) can be detected with reasonable efficiency. Although conventional Giemsa staining and electronic karyotyping cannot detect every chromosome aberration, it can detect a reasonable fraction of the stable aberrations as shown by comparison with the FISH-based method using the same study subjects. This study compares the frequency of total translocations and dicentrics in former plutonium workers using both the FISH – based technology (painting chromosome pairs 1, 4, and 12) including a pancentromeric DNA probe for dicentric analysis and electronic karyotyping using replicate slides from the same cell cultures.

The sample of plutonium workers and controls used in this study is a subset of the same groups used in the FISH – based study of plutonium workers.[1] The original study measured chromosome aberration rates in lymphocytes of 30 retired plutonium workers with combined internal and external radiation doses > 0.5 Sv and 21 control subjects having no history of occupational radiation exposure.[1] The subsets were chosen based on the availability of replicate slides to perform the Giemsa staining, since all of the original cases did not have surplus slides. The surplus, unstained slides were stored at room temperature and were subsequently stained with the conventional (solid) Giemsa staining

method. Karyotype screening was performed on 50 metaphase cells for each of 15 of the exposed workers and 15 of the unexposed, control workers. Thus a total of 750 karyotypes were constructed for each of the two study groups.

Results

The frequency of chromosome translocations and dicentric chromosomes detected by FISH and karyotype analysis is shown in Table 1. In the original FISH study, the unexposed control group (n=21) showed a total translocation frequency per genome equivalent of 4.00 ± 0.60 per 1,000 cells compared to 16.98 ± 2.05 per 1,000 cells for the exposed group (n=30)[1]. In the follow-up karyotype screening study, a subset of 15 workers from the original control group and 15 workers from the original exposed group showed a total translocation frequency per genome equivalent of 4.26 ± 0.72 and 22.08 ± 3.34 per 1,000 cells, respectively (Table 1). The increased frequency for the exposed sub-group of 15 workers may be related to the increase in the median bone marrow dose (168 vs. 710 mSv) for the subset of 15 exposed workers compared to the original 30 workers, but it is also well within the standard errors for both means.

Digital images illustrating the detection of chromosome aberrations by automated karyotype screening are shown in Fig. 1 (metaphase spread) and Fig. 2 (karyotype). Fig. 1 illustrates 46 chromosomes in a metaphase spread, as viewed in the microscope, showing a conspicuous abnormality (inversion) in a chromosome 1 (arrow). However, after the digital karyotype is constructed, additional chromosome aberrations in the cell become visible but are difficult to detect until after the karyotype is arranged using the International System for Cytogenetic Nomenclature (2005).[2] An inspection of the karyotype on the computer monitor reveals four additional chromosome aberrations which are involved in translocations (4q-; 9p+) and (15 q-; 15q+). Without an in depth examination of all the chromosome pairs in a karyotype format, it is unlikely the additional chromosome aberrations would have been detected. The presence of five chromosome aberrations in a single cell is consistent with an interpretation of exposure to

an alpha particle intersecting the cell's nucleus and releasing its energy at multiple sites affecting multiple chromosomes.

The mean translocation frequencies per genome equivalent for the exposed (n= 15) and control (n=15) groups measured by FISH analysis vs. karyotype screening is given in Table 1. For the control group, the mean total translocation frequency per 1,000 cells is 4.26 ± 0.72 and 4.00 ± 2.14 for the FISH method (Genome Equivalent [GE]) and karyotyping method, respectively. For the exposed group, the mean total translocation frequency per 1,000 cells is 22.08 ± 3.34 and 18.67 ± 4.96 for the FISH (GE) and karyotyping methods, respectively. The two methods of analysis are comparable, with karyotype screening providing an estimate within 85% of the FISH-based result.

A comparison of the mean dicentric chromosome frequencies per 1,000 cells for the exposed (n=15) and control (n=15) groups measured by FISH analysis and karyotype screening is also presented in Table 1. For the control group, the dicentric frequency per 1,000 cells is 0.09 ± 0.04 and 2.67 ± 1.82 for the FISH and karyotyping methods, respectively. For the exposed group, the dicentric frequency per 1,000 cells is 0.76 ± 0.20 and 13.33 ± 5.04 for the FISH and the karyotyping methods, respectively showing sharp discordance between the methods of analysis.

Discussion

The widespread use of ionizing radiation in medical, industrial, research and military settings has increased the risk of accidental human exposures. Following the terrorist attacks on the U.S., however, a far greater radiation concern has emerged since the risk of deliberate use of radiological and nuclear materials has increased dramatically. Chromosome biodosimetry is especially attractive where events are poorly characterized and physical dose measurements are not available such as in a terrorist attack. In this circumstance, lymphocytes can serve as biological dosimeters because of their high degree of radiation sensitivity, circulation throughout all organs, accessibility by routine venipuncture, ease of culture in vitro, long life span and thus their ability to integrate exposure over time. The cytogenetic analysis of blood cells for purposes of dose assessment is likely to play a key role in the medical management of potentially

irradiated individuals following a significant radiological event. The merits of this method have developed steadily over four decades and have consistently shown that chromosomal DNA damage increases as a function of the absorbed dose and thus can be used to estimate energy deposition in the body in the absence of physical dosimeters. This is accomplished most effectively by quantifying the frequency of dicentric chromosomes in metaphase cells and comparing the frequency to a standardized dose-effect or calibration curve. A significant limitation of this method is the laborious and time consuming nature of the task which can be reduced by automation, including the finding and analysis of metaphase cells. State-of-the-art hardware coupled with innovative software algorithms involving a slide scanning platform which automatically controls specimen identification by bar coding and tracking, slide movement, focusing, cell image acquisition, creation of image galleries, enumeration of dicentrics and laboratory networking should provide the capability to process a large number of samples in a mass casualty event.

In summary, it appears prudent to further develop, test and validate the cytogenetic protocol as described in this promising study. This limited study supports the efficacy of automated karyotyping as a method to detect both stable (translocations) and unstable (dicentrics) chromosome aberrations and may well augment the dicentric assay by including a wider spectrum of chromosomal damage. This is especially true when examining individuals who were exposed to ionizing radiation from internal emitters decades prior to cytogenetic examination.

Conclusions

The results showed that based on screening a total of 750 karyotypes for each group (15 control subjects and 15 exposed subjects), automated karyotyping revealed a significant increase in both stable and unstable chromosome aberrations in the group with a history of occupational exposure to plutonium. The mean frequency of abnormal karyotypes (i.e. those with one or more chromosomes with structural aberrations) was 4.4% (range: 2-8) in the control group and 16.8% (range: 4-30) in the exposed group. Thus karyotyping as

few as 50 cells per subject was capable of detecting a radiation effect in former plutonium workers based on an analysis of the means.

Although karyotype screening of conventional Giemsa stained chromosomes cannot detect all chromosome aberrations, the overall detection rate for total translocations in the exposed and control groups was, on average, within 85% (exposed) and 94% (controls), respectively, of the value obtained using the FISH method .

The automated, on-screen karyotyping method detected a significantly higher frequency of dicentric chromosomes for both the exposed (13.33 per 1,000 cells) and control (2.67 per 1,000 cells) groups when compared to the FISH method (0.76 per 1,000 cells and 0.09, per 1,000 cells) for exposed and control groups, respectively (Table 1). Since the background frequency of dicentric chromosomes is 1-2 per 1,000 cells,[5] the FISH method as applied in this study, appears to significantly underestimate the true frequency. It is unknown why this occurred.

In summary, results of this cytogenetic study on former plutonium workers with chronic, low-dose exposure to radiation, supports earlier work on a larger number of A-bomb survivors showing that solid Giemsa staining, when accompanied by karyotype analysis, can significantly increase the efficiency for detecting radiation-induced translocations. Additionally, the electronic karyotyping of metaphase spreads is a useful and time saving method for analyzing chromosome aberrations.

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